Rhamnolipid biosurfactants production by strains of *Pseudomonas aeruginosa* using low cost raw materials

K.S.M. Rahman*, Thahira J.Rahman, Stephen McClean †

Roger Marchant and Ibrahim M. Banat

Biotechnology Research Group, School of Biological and Environmental Sciences, University of Ulster, Coleraine –BT52 1SA , Northern Ireland, UK

† *Pharmaceutical-Biotechnology Research Group, School of Biomedical Sciences, University of Ulster, Coleraine –BT52 1SA , Northern Ireland, UK*

* Corresponding author present address

Dr Pattanathu K.S.M. Rahman

Chemical and Bioprocess Engineering Group
School of Science and Engineering
Teesside University, Middlesbrough - TS1 3BA
Teesvalley, United Kingdom.

Tel: +44-1642-384669
Email: p.rahman@tees.ac.uk

Abstract

This study was aimed at the development of economical methods for higher yields of biosurfactant by suggesting the use of low cost raw materials. Two oil-degrading strains, *Pseudomonas aeruginosa* GS9-119 and DS10-129 were used to optimize substrate for maximum rhamnolipid production. Among the two strains, the latter produced a maximum of 4.31, 2.98 and 1.77 g/L rhamnolipid biosurfactant using soybean oil, safflower oil and glycerol, respectively. The yield of biosurfactant steadily increased even after the bacterial cultures reached the stationary phase of growth. Characterization of rhamnolipids using Mass Spectrometry revealed the presence of dirhamnolipids (Rha-Rha-C_{10}-C_{10}). Emulsification activity of the rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* DS10-129 was greater than 70% using all the hydrocarbons tested including xylene, benzene, hexane, crude oil, kerosene, gasoline and diesel. *Pseudomonas aeruginosa* GS9-119 emulsified only hexane and kerosene to that level.

Introduction

Rhamnolipid biosurfactants are surface-active compounds produced by *Pseudomonas aeruginosa*. They can reduce surface tension, stabilise emulsions, promote foaming and are generally non-toxic, non-hazardous and biodegradable [1]. Due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, effectiveness under extreme conditions in small quantities, production on renewable sources and potential applications in environmental protection, biosurfactants are gaining prominence over chemical surfactants. In addition, the valuable carbohydrate moiety, rhamnose of the rhamnolipid biosurfactant is used for the transport of insoluble drugs in humans and acts as precursor for high – quality flavour components.

Biosurfactants enhance emulsification of hydrocarbons, and therefore have the potential to solubilize hydrocarbon contaminants and increase their availability for microbial degradation. Hence, biosurfactant producing microorganisms may play an important role in the accelerated bioremediation of hydrocarbon contaminated sites [2, 3]. Due to the high cost of remediation processes and other potential applications, the need for increasing the yields of biosurfactants is inevitable. Although the potential for biosurfactant production is determined by the genetics of the microorganisms, other factors like environmental conditions and nature of the substrate also influence the level of expression. Hence, optimization of these conditions may lead to high and safe biosurfactant production.

Most of the studies to date describe biosurfactant production by bacteria grown on hydrocarbons but a few have reported biosurfactants produced by bacteria growing on carbohydrates [4]. Most of the world's total oil and fat production is derived from plants. Hence these hydrophobic substrates may be used for bulk production of rhamnolipid biosurfactants. There are reports regarding the production of biosurfactants from different substrates like glycerol [5], soybean oil [6], olive oil [7], corn oil [8], canola oil [9], ethanol

[10], sucrose and whey [11] by different strains of *Pseudomonas aeruginosa*. Use of water-miscible wastes like molasses, whey and distillery wastes, wastewater from olive oil processing has also been reported [11,12,13,14].

In our earlier studies *Pseudomonas aeruginosa* GS9-119 and *Pseudomonas aeruginosa* DS10-129 produced 4.3 g/L and 7.5 g/L of crude biosurfactant respectively, when grown on glucose + glycerol medium [15, 16]. Since they were found to be efficient biosurfactant producers, the present study aimed at investigating biosurfactant production using different substrates such as soybean oil, safflower oil and glycerol and to quantify and characterize the pure rhamnolipid biosurfactant produced by them.

Materials and Methods

Screening of samples. Soil samples were collected from gasoline and diesel spilled gas station soil for the isolation of oil degrading biosurfactant-producing bacteria. The samples were collected in pre-sterilized glass bottles and transported to the laboratory for analyses. An enrichment and isolation procedure for bacteria was carried out using the serial dilution-agar plating technique on nutrient agar medium (HIMEDIA). Two pure cultures capable of growth on mineral salt media [17] containing crude oil as the only carbon source were selected for further testing.

Identification of bacteria. The isolated bacterial cultures were characterized depending on their morphological and biochemical characteristics [18].

Batch fermentation using different substrates. Series of 250 mL conical flasks containing 100 mL of mineral salts medium (MSM) and at 2 g/L glucose were prepared and the pH was maintained at 7.5. The flasks were inoculated with one of the strains and incubated in a shaker at 200 rpm at 30^{o}C. Additional substrate was added as safflower oil, soybean oil or glycerol at 2 g/L after 46, 144 and 192 h to each of the respective flasks and reincubated.

Samples were withdrawn at intervals for analyses of biomass, biosurfactant and surface tension estimation.

Biomass estimation. Ten millilitre of the sample was withdrawn from the culture flasks at every 24 h interval for 288 h and was centrifuged at 4000 rpm for 20 minutes. The pellet containing cells was dried in an oven at 110°C and the dry weight of the cells was calculated.

Biosurfactant estimation. One millilitre of cell free culture broth was added to 4.5 mL of dilute sulfuric acid (6:1 v/v) and mixed thoroughly. The mixture was heated at 100^{o}C for 10 minutes and cooled down to room temperature. To the mixture, 0.1 mL of freshly prepared 3% solution of thioglycolic acid was added and incubated in darkness for three hours. Absorbance was measured at 400 and 430nm spectrophotometrically. Rhamnolipid concentration was calculated using the formula: RL = [54.18 (A_{400}-A_{430}) – 1.49] F. Where, A_{400} and A_{430} are absorbance at 400nm and 430nm respectively and F is the dilution factor. A standard curve prepared using different concentrations of L-rhamnose (Sigma) was used to determine the rhamnolipid concentration [19].

Surface tension measurements. Surface tension of the culture broth was measured using a KRUSS KIOT Tensiometer (KRUSS, Optische – Mechanische Werkstatten, Hamburg, Germany) equipped with a 6 cm De Nuoy platinum ring. To increase the accuracy, average of triplicates was used for the study.

Characterization of Rhamnolipid using Mass spectrometry. Rhamnolipid fraction from culture free supernatant was extracted by adding equal volume of Chloroform: Methanol (2:1) solvent mixture and mixed thoroughly. Then the organic layer was separated using separatory funnel, air dried and dissolved in methanol. Mass spectrometry characterization and detection of the rhamnolipid fractions under investigation was performed using an

LCQ^{TM} quadrupole ion-trap mass spectrometer (Finnigan MAT, San Jose, California, USA) utilizing electrospray ionization (ESI). Standard solutions and samples under investigation were infused into the mass spectrometer at a flow rate of 10 μl/min. In the ESI source nitrogen sheath and auxiliary gas flows were maintained at 50 and 5 respectively and refer to arbitrary values set by the software. The heated capillary temperature was 250^{o}C and the spray voltage set to 5kV. Negative ion mode was used throughout and scans initiated over the 50-2000 m/z range.

Determination of Emulsification activity. Emulsification activity (E24) was determined by the addition of equal volumes of hydrocarbon and cell free culture broth (bacteria grown in the presence of soybean oil at 288h), mixing with a vortex for 2 minutes and leaving to stand for 24 h. The emulsification activity was determined as the percentage of height of emulsified layer (mm) divided by total height of the liquid column (mm) [20].

Results and Discussion

Among 130 oil degrading bacterial isolates from hydrocarbon polluted environments [21], two strains of *Pseudomonas* were found to efficiently utilize crude oil and produce biosurfactant. Similar enrichment procedures using oil-contaminated samples have been reported as sources of biosurfactant producing, oil degrading bacteria [22, 23]. Detailed biochemical characterization of the isolates showed (Table 1) that they were two different strains of *Pseudomonas aeruginosa* and were designated the names GS9-119 and DS10-129. Mineral salts medium used for growing biosurfactant producers was initially supplemented with 2 g/L glucose to initiate biomass production. This was followed by the addition of various substrates like safflower oil, soybean oil or glycerol to test their influence on biosurfactant production. Among the three substrates tested, DS10-129

produced a maximum of 4.31 g/L rhamnolipid with soybean oil at 288h followed by 2.98 g/L and 1.77 g/L on safflower oil and glycerol, respectively. The surface tension of the cell free culture broth were reduced to 28, 30 and 29.1 mN/m, respectively (Figure 1A). For GS9-119 maximum biosurfactant production was 1.75 g/L on soybean oil at 288h while on glycerol and safflower oil were 1.66 g/L and 1.06 g/L respectively (Figure 1B). Maximum reduction in surface tension was observed with soybean oil, followed by glycerol and safflower oil.

In our study 4.3 g/L rhamnolipid was produced from 6 g/L of soybean oil (0.716 g/g product yield), which was higher when compared to 0.625 gram rhamnolipid per gram of soybean oil reported by Lang and Wullbrandt [6]. Earlier reports [5, 24] revealed about 2.2–5.8 g/L (10-20%) of rhamnolipid production using 20-30 g/L of glycerol by two different strains of *Pseudomonas aeruginosa*. But, in our present study using 6 g/L of glycerol, GS9-119 and DS10-129 produced about 1.66 g/L (0.276 g/g) and 1.77 g/L (0.295 g/g) of rhamnolipid respectively. Rhamnolipid production – Substrate (R/S) conversion factor was higher in the present findings than the previous reports, which clearly depicts the efficiency of biosurfactant production by our isolates. However we have not tested the ability of our isolates to grow to high biomass density and high biosurfactant concentrations comparable to previously reported results by Lang and Wullbrandt [6].

About 0.97 – 2.7 g/L of biosurfactant production by different strains of *Pseudomonas aeruginosa* using glucose and waste fry oil as carbon source had been reported [25, 26, 27]. When compared to the above reports, DS10-129 showed higher quantity of rhamnolipid production with soybean oil. 46 g/L (61%) of rhamnolipid production with 75 g/L of corn oil by *Pseudomonas aeruginosa* UI 29791, 10 g/L (50%) of biosurfactant production with 20 g/L of olive oil by *Pseudomonas aeruginosa* 44T1 and 24.3 g/L (40.5%) of rhamnolipid production using 60 g/L of canola oil by *Pseudomonas aeruginosa* UW1 have been reported

[7,8,9]. Arino et al [5] reported higher yield of biosurfactant with glycerol than with hydrophobic carbon sources. But in our studies DS10-129 showed higher biosurfactant productions with soybean oil and safflower oil than glycerol. An increase in biomass was observed with soybean oil and safflower oil than glycerol with an increase in biosurfactant production. We found an increase in biosurfactant production even after the bacterial growth had reached stationary phase. This may be because of the production of biosurfactant as a secondary metabolite. Biosurfactant production accompanying the growth of oil degrading bacteria may help in the adherence of the cells to the substrate molecules and metabolize them [23].

Rhamnolipid biosurfactant mediated surface tension reduction of 28 mN/m by *Pseudomonas* sp. DSM-2874, 25 mN/m by *Pseudomonas aeruginosa* 44T1 and 28 mN/m by *Pseudomonas aeruginosa* BOP100 were previously reported [28, 29, 30]. These findings supported our results obtained with DS10-129. Reduction of surface tension measurements observed in our experiments indicated the production of surface active compounds like rhamnolipid.

Mass spectrometry using electrospray ionization is an efficient and simple method to characterize rhamnolipids produced by *Pseudomonas aeruginosa*. Deziel et al [31] has reported about different rhamnolipid species produced by *Pseudomonas aeruginosa* 57RP with mannitol and naphthalene as carbon source. They have observed the predominance of dirhamnolipids (Rha-Rha-C_{10}-C_{10}) in their analysis at 649 m/z. During our study, in the spectrum of rhamnolipids obtained with GS9-119 and DS10-129, dirhamnolipids (Rha-Rha-C_{10}-C_{10}) was the predominant rhamnolipid produced (Figure 2). As exemplified by the previous report, most of the ions above 447 m/z were rhamnolipid pseudomolecular $(M-H)^-$ ions and ions less than 507 m/z were fragment ions produced by cleavage [32].

Strain DS10-129 emulsified more than 70% of all the hydrocarbons tested such as xylene, benzene, hexane, crude oil, kerosene, gasoline, diesel and crude oil, whereas GS9-119 emulsified only hexane and kerosene to that extent (Figure 3). There were reports [20,23] regarding emulsification of hydrocarbon substrates such as toluene, hexane, sunflower oil, kerosene and crude oil. In our previous report, *Pseudomonas* sp. MR–3 emulsified only 31.70% of crude oil [33]. But in our present findings both the strains of *Pseudomonas aeruginosa* showed higher emulsification activity (58-73%).

Conclusion

Our results indicated that the above strains of *Pseudomonas aeruginosa* were efficient in rhamnolipid biosurfactant production and hydrocarbon emulsification. Soybean oil supplements increased the biomass and rhamnolipid production to several folds than safflower oil and glycerol. These results suggested the use of the low cost renewable carbon source like soybean oil for the biosurfactant production, which could be applied for bioremediation of hydrocarbon contaminated sites and enhanced oil recovery.

Acknowledgements

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References

(1) Banat, I.M.; Makkar, R.S.; Cameotra, S.S. Potential commercial applications of microbial surfactants. *Appl. Microbiol. Biotechnol*. **2000**, *53:* 495-508.

(2) Rosenberg, E.; Ron, E.Z. High and low molecular mass microbial surfactants. *Appl. Microbiol. Biotechnol*. **1999**, *52*: 154-162.

(3) Del' Arco, J.P.; de Franka, F.P. Influence of oil contamination levels on hydrocarbon biodegradation in sandy sediment. *Environ. Pollut*. **2001**, *110*: 515-519.

(4) Haferburg, D.; Hommel, R.; Claus, R.; Kleber, H.P. Extra-cellular microbial lipids as biosurfactants. *Adv. Biochem. Eng. Biotechnol*. **1986**, *33*: 53-93.

(5) Arino, S.; Marchal, R.; Vandecasteele, J.P. Identification and production of rhamnolipidic biosurfactant by a *Pseudomonas* species. *Appl. Microbiol. Biotechnol*. **1996**, *45*: 162-168.

(6) Lang, S.; Wullbrandt, D. Rhamnose lipids – biosynthesis, microbial production and application potential. *Appl. Microbiol. Biotechnol*. **1999**, *51*: 22-32.

(7) Manresa, M.A.; Bastida, J.; Mercade, M.E.; Robert, M.; Andres, J.D.; Espuny, M.J.; Guinea, J. Kinetics studies on surfactant production by *Pseudomonas aeruginosa* 44T1. *J. Ind. Microbiol*. *Biotechnol*. **1991**, *5*: 25-32.

(8) Linhardt, R.J.; Bakhit, R.; Daniels, L.; Mayerl, F.; Pickenhagen, W. Microbially produced rhamnolipid as a source of rhamnose. *Biotechnol. Bioeng*. **1989,** *33*: 365-368.

(9) Sim, L.; Ward, O.P.; Li, Z.Y. Production and characterization of biosurfactant from *Pseudomonas aeruginosa* UW-1. *J. Ind. Microbiol. Biotechnol*. **1997**, *19*: 232-238.

(10) Matsufuji, M.; Nakata, K.; Yoshimoto, A. High production of rhamnolipids by *Pseudomonas aeruginosa* growing on ethanol. *Biotechnol. Lett*. **1997**, *19*: 1213-1215.

(11) Babu, P.S.; Vaidya, A.N.; Bal, A.S.; Kapur, R.; Juwarkar, A.; Khanna, P. Kinetics of biosurfactant production by *Pseudomonas aeruginosa* strain BS2 from industrial waste. *Biotechnol. Lett*. **1996**, *18*: 263 – 268.

(12) Mercade, M.E.; Monleon, L.; de Andres, C.; Rodon, I.E.M.; Espuny, M.J.; Manresa, A. Screening and selection of surfactant-producing bacteria from waste-lube oil. *J. Appl. Bacteriol.* **1996**, *81*: 161-166.

(13) Patel, J.L.; Desai, A.J. Biosurfactant production from *Pseudomonas aeruginosa* GS3. *Lett. Appl. Microbiol*. **1997**, *25*: 91-94.

(14) Daniel, H.J.; Otto, R.T.; Binder, M.; Reussm, M.; Syldatk, C. Production of sophorolipids from whey: development of a two-stage process with *Cryptococcus curvatus* ATCC 20509 and *Candida bombicola* ATCC 22214 using deproteinized whey concentrates as substrates. *Appl. Microbiol. Biotechnol*. **1999**, *51*: 40-45.

(15) Rahman, K.S.M.; Banat, I.M.; Thahira-Rahman, J.; Thayumanavan, T.; Lakshmanaperumalsamy, P. Bioremediation of Gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant. *Bioresource Technol.* **2002**, *81*: 25-32.

(16) Rahman, K.S.M.; Thahira-Rahman, J.; Lakshmanaperumalsamy, P.; Marchant, R.; Banat, I.M. Emulsification potential of bacterial isolates with a range of hydrocarbon substrates. *Acta Biotechnol*. (Submitted) **2002**.

(17) Kennedy, R.S.; Finnerty, W.R.; Sudarsanan, K.; Young, R.A. Microbial assimilation of hydrocarbons - the fine structure of a hydrocarbon oxidising *Acinetobacter* sp. *Arch. Microbiol.* **1975**, *102*: 75-83.

(18) Holt, J.G.; Kreig, N.R.; Sneath, P.H.A.; Stanely, J.T.; Williams, S.T. *Bergey's Manual of Determinative Bacteriology*, 9th edition, Williams and Wilkins Publishers, Maryland **1994**.

(19) Zhang, Y.; Miller, R.M. Enhanced octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid surfactant (biosurfactant). *Appl. Environ. Microbiol*. **1992**, *58*: 3276-3282.

(20) Iqbal, S.; Khalid, Z.M.; Malik, K.A. Enhanced biodegradation and emulsification of crude oil and hyperproduction of biosurfactants by a gamma ray-induced mutant of *Pseudomonas aeruginosa*. *Lett. Appl. Microbiol*. **1995**, *21*: 176-179.

(21) Rahman, K.S.M.; Thahira-Rahman, J.; Lakshmanaperumalsamy, P.; Banat, I.M. Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. *J. Basic Microbiol.* **2002**, *42*:286-293.

(22) Fleck, L.C.; Bicca, F.C.; Ayub, M.A.Z. Physiological aspects of hydrocarbon emulsification, metal resistance and DNA profile of biodegrading bacteria isolated from oil polluted sites. *Biotechnol. Lett*. **2000**, *22*: 285-289.

(23) Bharathi, S.; Vasudevan, N. Utilisation of hydrocarbons by *Pseudomonas fluorescens* isolated from a petroleum-contaminated soil. *Environ. Int*. **2001**, *26:* 413-416.

(24) Ochsner, U.A.; Reiser, J.; Fiechter, A.; Witholt, B. Production of *Pseudomonas aeruginosa* rhamnolipid biosurfactants in Heterologous hosts. *Appl. Environ. Microbiol*. **1995**, *61*: 3503-3506.

(25) Guerra-Santos, L.H.; Kappeli, O.; Fiechter, A. Dependence of *Pseudomonas aeruginosa* continuous culture biosurfactant production on nutritional and environmental factors. *Appl. Microbiol. Biotechnol*. **1986**, *24*: 443-448.

(26) Haba, E.; Espuny, M.J.; Busquets, M.; Manresa, A. Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oil. *J. Appl. Microbiol*. **2000**, *88*: 379-387.

(27) Dubey, K.; Juwarkar, A. Distillery and curd whey wastes as viable alternative sources for biosurfactant production. *World J. Microbiol. Biotechnol*. **2001**, *17:* 61-69.

(28) Syldatk, C.; Lang, S.; Wagner, F.; Wrey, V.; Witte, L. Chemical and physical characterization of four interfacial-active rhamnolipids from Pseudomonas sp. DSM 2874 grown on n-alkanes. *Z-Naturforsch*. **1985**, *40*: 51-60.

(29) Parra, J.L.; Guinea, J.; Manresa, M.A.; Robert, M.; Mercade, M.E.; Comelles, F.; Bosch, M.P. Chemical characterization and physico-chemical behaviour of biosurfactants. *J. Am. Oil Chem. Soc.* **1989**, *66*: 141-145.

(30) Ishigami, Y.; Gama, Y.; Ishii, F.; Choi, Y.K.; Colloid chemical effect of polar head moities of a rhamnolipid- type biosurfactant. *Langmuir*. **1993**, *9*: 1634-1636.

(31) Deziel, E.; Lepine, F.; Dennie, D.; Boismenu, D.; Mamer, O.A.; Villemur, R. Liquid chromatography/mass spectrophotometry analysis of mixtures of rhamnolipids produced by *Pseudomonas aeruginosa* 57RP grown on mannitol or naphthalene. *Biochim. Biophys. Acta*. **1999**, *1440*: 244-252.

(32) Deziel, E.; Lepine, F.; Milot, S.; Villemur, R. Mass spectrophotometry monitoring of rhamnolipids from a growing culture of *Pseudomonas aeruginosa* 57RP. *Biochim. Biophys. Acta.* **2000**, *1485*: 145-152.

(33) Rahman, K.S.M.; Vasudevan, N.; Lakshmanaperumalsamy, P. Enhancement of biosurfactant production to emulsify different hydrocarbons. *J. Environ. Pollut*. **1999**, *6*: 85-93.

Table 1. Biochemical characterization

Characteristics	GS9-119	DS10-129	Characteristics	GS9-119	DS10-129
Cell Morphology			**Production of acid from**		
Gram staining	Negative	Negative	Raffinose	-	-
Shape	Rods	Rods	Maltose	-	-
Motility	+	+	Ribose	+	-
Sporulation	-	-	Trehalose	-	-
Pyocyanin Production	+	+	Sorbitol	-	-
Growth at			Glycerol	-	-
20 ^{o}C	-	+	Rhamnose	-	-
30 ^{o}C	+	+	Arabinose	-	+
40 ^{o}C	+	+	Cellobiose	-	-
50 ^{o}C	+	+	Glycogen	+	+
Biochemical Reactions			**Hydrolysis of**		
Indole production test	-	-	Starch	+	+
Methyl red test	-	-	Cellulose	+	+
Voges Proskauer test	-	-	Casein	+	+
Citrate utilization test	+	+	Gelatin	+	+
Oxidase test	+	-	Urea	-	+
Catalase test	+	+	Tween 80	+	+
Production of acid from			**Utilization of**		
Glucose	+	+	Amylopectin	+	+
Mannose	-	-	Cellulose	+	+
Fructose	-	-	Carboxymethyl cellulose	-	-
Xylose	+	-	Sodium acetate	+	+
Lactose	-	-	Sodium formate	+	+
Sucrose	-	-	Myo-inositol	+	+
Galactose	+	+	Crude oil	+	+

- Negative result
+ Positive result

Figure Legends

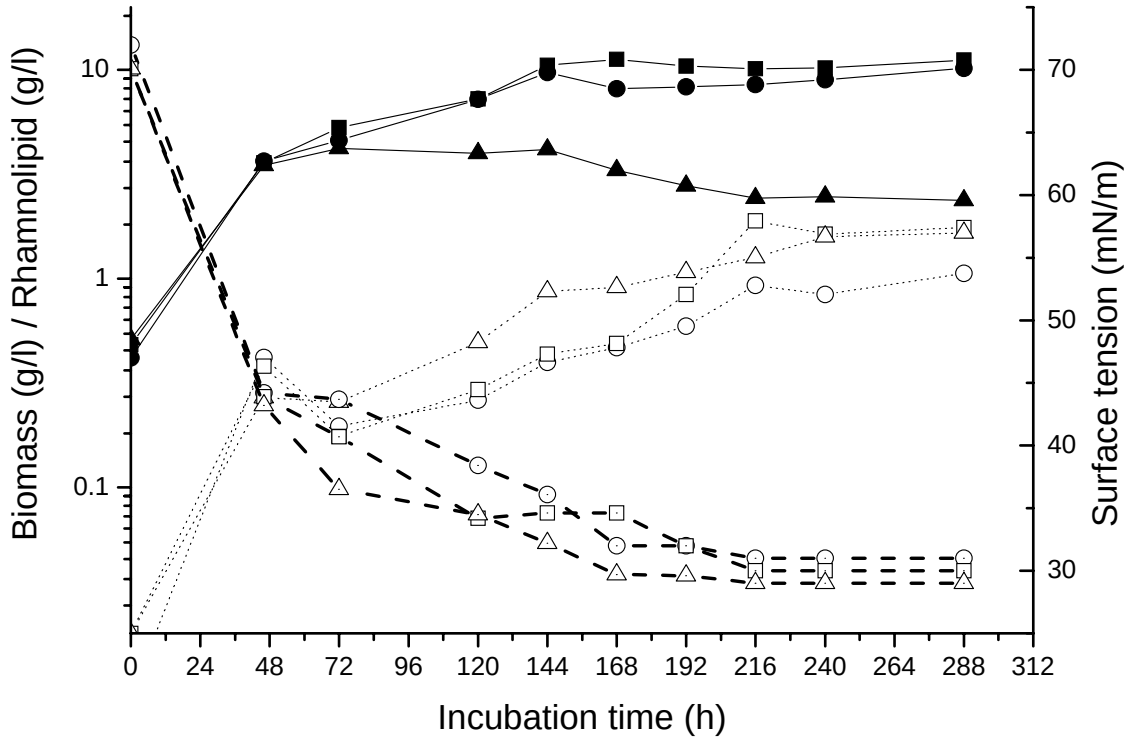
Figure 1A. Biomass (—), Rhamnolipid production (·····) and Surface tension (----) reduction using safflower oil (●), Soybean oil (■) Glycerol (▲) at regular time intervals by *Pseudomonas aeruginosa* GS9-119

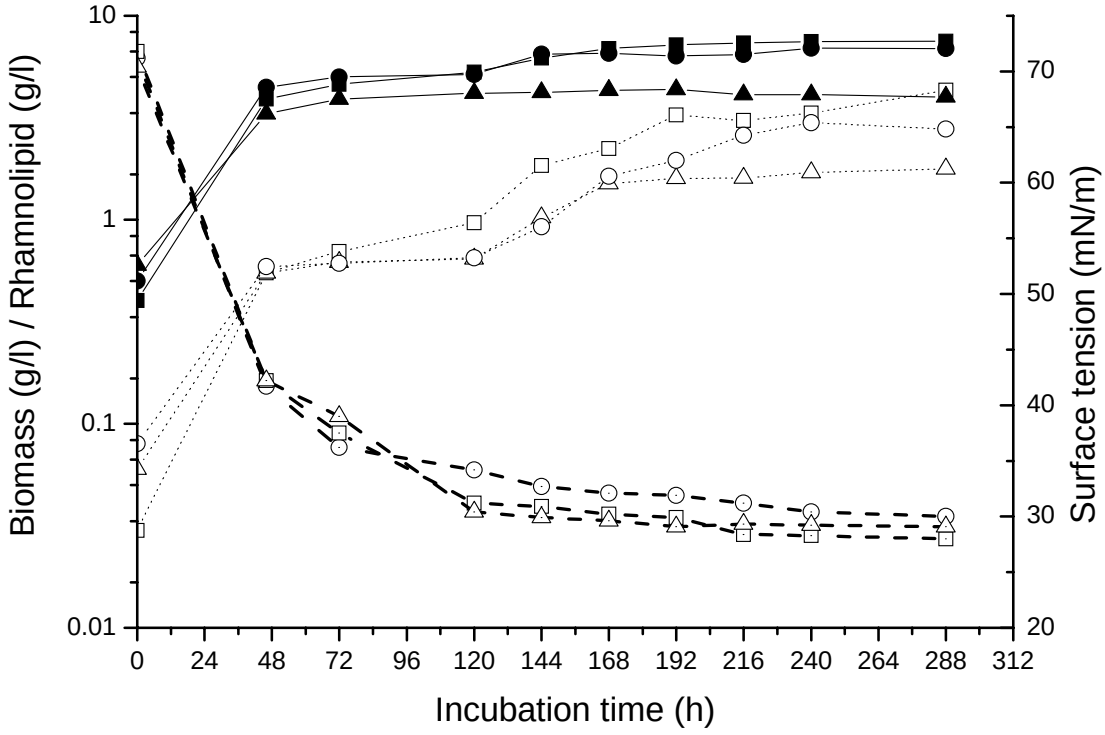
Figure 1B. Biomass (—), Rhamnolipid production (·····) and Surface tension (----) reduction using safflower oil (●), Soybean oil (■) Glycerol (▲) at regular time intervals by *Pseudomonas aeruginosa* DS10-129

Figure 2A. Mass spectrum of rhamnolipids produced by *Pseudomonas aeruginosa* GS9-119 using soybean oil as substrate

Figure 2B. Mass spectrum of rhamnolipids produced by *Pseudomonas aeruginosa* DS10-129 using soybean oil as substrate

Figure 3. Emulsification activity of rhamnolipid on various hydrocarbons


Biomass (g/l) / Rhamnolipid (g/l)
Surface tension (mN/m)
Incubation time (h)
10
1
0.1
70
60
50
40
30
0
24
48
72
96
120
144
168
192
216
240
264
288
312


Biomass (g/l) / Rhamnolipid (g/l)
Surface tension (mN/m)
Incubation time (h)
10
1
0.1
0.01
70
60
50
40
30
20
0
24
48
72
96
120
144
168
192
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240
264
288
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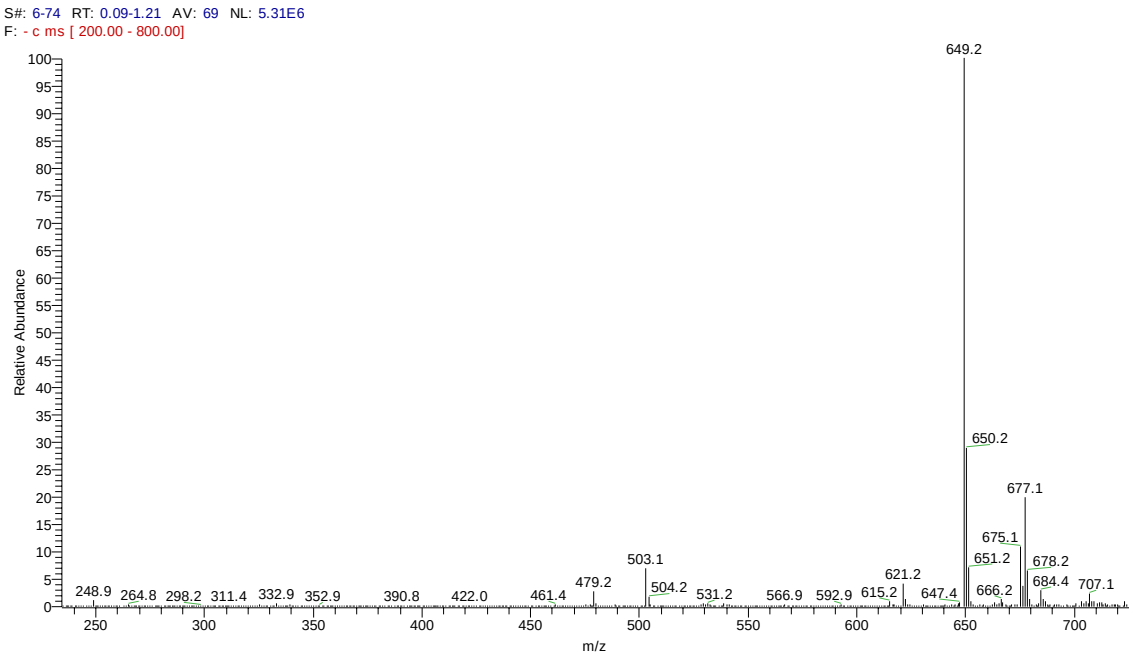
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Relative Abundance
m/z
649.2
650.2
677.1
675.1
651.2
678.2
684.4
707.1
503.1
504.2
479.2
621.2
615.2
647.4
666.2
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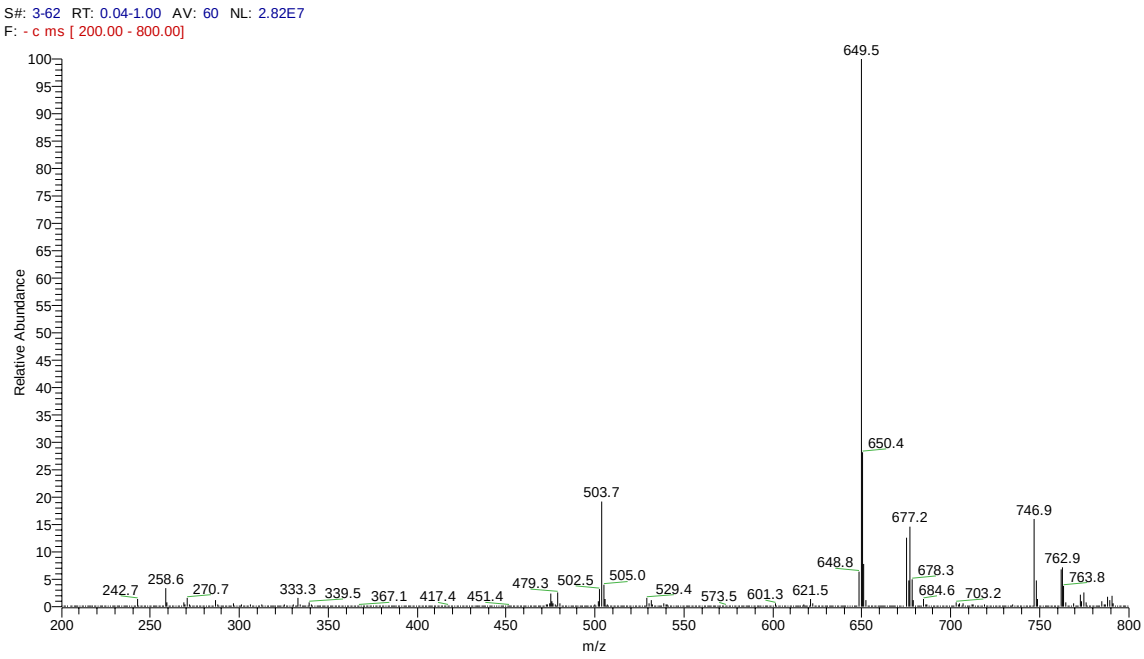
566.9
592.9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Relative Abundance
m/z
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650.4
503.7
677.2
746.9
762.9
763.8
678.3
684.6
703.2
648.8
621.5
601.3
573.5
529.4
505.0
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479.3
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367.1
339.5
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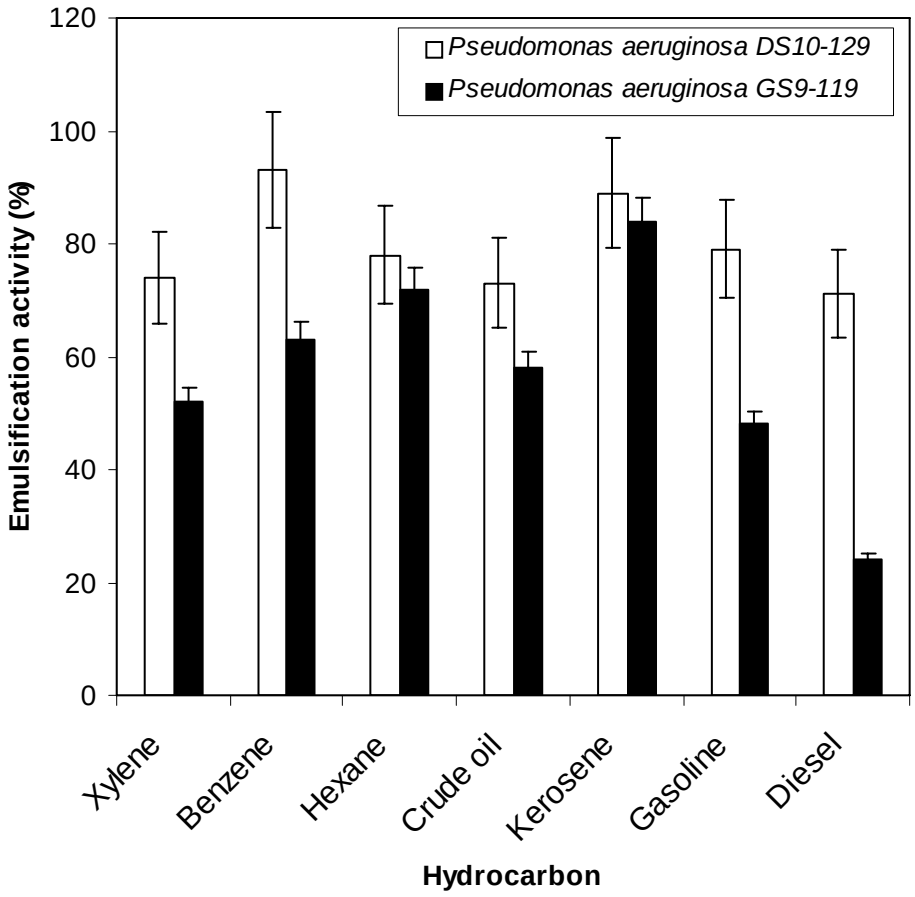
258.6
242.7


120
100
80
60
40
20
0
Emulsification activity (%)
Pseudomonas aeruginosa DS10-129
Pseudomonas aeruginosa GS9-119
Xylene
Benzene
Hexane
Crude oil
Kerosene
Gasoline
Diesel
Hydrocarbon